Occurrence, Identification, and Antimicrobial Resistance Profiles of Extended-Spectrum and AmpC b-Lactamase-Producing Enterobacteriaceae from Fresh Vegetables Retailed in Gauteng Province, South Africa

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Abstract

Extended-spectrum b-lactamase (ESBL) and AmpC b-lactamase-producing *Enterobacteriaceae* are no longer restricted to the health care system, but represent increased risks related to environmental integrity and food safety. Fresh produce has been increasingly reported to constitute a reservoir of multidrug-resistant (MDR) potential human pathogenic *Enterobacteriaceae*. This study aimed to detect, identify, and characterize the antimicrobial resistance of ESBL/AmpC-producing *Enterobacteriaceae* isolates from fresh vegetables at point of sale. Vegetable samples (spinach, tomatoes, lettuce, cucumber, and green beans; *n*=545) were purchased from retailers in Gauteng, the most densely populated province in South Africa. These included street vendors, trolley vendors, farmers' market stalls, and supermarket chain stores. Selective enrichment, plating onto chromogenic media, and matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDITOF MS) confirmation of isolate identities showed that 17.4% (95/545) vegetable samples analyzed were contaminated with presumptive ESBL/AmpC-producing *Enterobacteriaceae.* Dominant species identified included *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter asburiae*, and *Klebsiella pneumoniae*. Phenotypic antibiotic resistance analysis showed that 96.1% of 77 selected isolates were MDR, while resistance to aminoglycoside (94.8%), chloramphenicol (85.7%), and tetracycline (53.2%) antibiotic classes was most prevalent. Positive phenotypic analysis for ESBL production was shown in 61 (79.2%) of the 77 isolates, and AmpC production in 41.6% of the isolates. PCR and sequencing confirmed the presence of b-lactamase genes in 75.3% isolates from all vegetable types analyzed, mainly in *E. coli*, *Enterobacter* spp., and *Serratia* spp. isolates. CTXM group 9 (32.8%) was the dominant ESBL type, while EBC (24.1%) was the most prevalent plasmidic type AmpC b-lactamase. Our findings document for the first time the presence of MDR ESBL/AmpC-producing *Enterobacteriaceae* in raw vegetables sold at selected retailers in Gauteng Province, South Africa.

Keywords: antibiotic resistance, fresh produce, food safety

Introduction

Extended-spectrumb-lactamase (ESBL)- and AmpC- spectrum penicillins and cephalosporins, their presence in producing *Enterobacteriaceae* have increased in occur- *Enterobacteriaceae* is of clinical and epidemiological rence globally in health care systems, agroecosystems, and fresh produce, due to the widespread use of broadspectrum antibiotics (Ye *et al.*, 2017). Dissemination of these antimicrobial-resistant microorganisms has been identified as one of the six main antibiotic resistance (AR)–related health risks globally (WHO, 2015). If infection by ESBL/ AmpC-producing *Enterobacteriaceae* occurs, treatment options become limited as a result of expanded AR of the corresponding isolates (Freitag *et al.*, 2018). Since ESBL/AmpC b-lactamases are capable of inactivating broad-

importance (Kolar *et al.*, 2010). Clinically important ESBLproducing *Enterobacteriaceae* have been reported in different South African (SA) provinces (Eastern Cape [Vasaikar *et al.*, 2017]; Western Cape [Peirano *et al.*, 2011]; KwaZuluNatal [Mahomed and Coovadia, 2014]; and Gauteng Province [Ehlers *et al.*, 2009]). In 53 clinical isolates from Gauteng, ESBL gene prevalence was reported in 87% (Ehlers *et al.*, 2009).

ESBLs, classified as Ambler class A enzymes, include TEM-, SHV- and CTX-M-type enzymes (O¨ stholm, 2014; Ghafourian *et al.*, 2015). More than 200 TEM and SHV variants have been documented, while 90 different enzymes within the CTX-M type have been described (O¨ stholm, 2014). Class A enzymes hydrolyze ampicillin and extendedspectrum cephalosporins (Ghafourian *et al.*, 2015). AmpC blactamases, classified as class C enzymes, are

resistant to additional b-lactams, that is, cephamycins, and are not influenced negatively by class A enzyme inhibitors (Jacoby, 2009; Njage and Buys, 2017). Plasmid-mediated AmpC (pAmpC)-producing strains are distinguished from chromosomal AmpC since they are often not inducible (Mezzatesta *et al.*, 2012). Six families of pAmpC-b-lactamases, including
CIT, FOX, MOX, DHA, EBC, and ACC, have been described, with DHA, CMY (CIT family member), and FOX most commonly detected (Thomson, 2010). Co-occurrence of b-lactamase enzymes, especially AmpC b-lactamases and ESBLs, is common (Thomson, 2010).

Salmonella spp., pathogenic *Escherichia coli*, and *Shigella* spp. have been implicated in foodborne disease outbreaks, while *Klebsiella pneumoniae*, *Serratia marcescens*, *Citrobacter freundii*, and *Enterobacter* spp. are regarded as opportunistic human pathogenic bacteria (Baylis *et al.*, 2011). The presence of ESBL/AmpC-producing *Enterobacteriaceae* on fresh produce has been studied worldwide (Kim *et al.*, 2015; Nu¨esch-Inderbinen *et al.*, 2015; Zurfluh *et al.*, 2015).

Transfer of multidrug-resistant (MDR) *Enterobacteriaceae* onto fresh produce occurs through the use of contaminated irrigation water or during production via animal manure (van Hoek *et al.*, 2015). Subsequent transfer to humans can happen through consumption of raw vegetables, potentially impacting consumer health negatively (Ye *et al.*, 2017). Concomitantly AR genes can easily be transferred to commensal bacteria that typically colonize the human gut.

Fresh vegetables produced in SA are retailed nationally and to the South African Development Community (SADC) countries, Swaziland, the UK, Middle East, and Asian markets (DAFF, 2012a, b, 2016). Current knowledge regarding the occurrence of ESBL/AmpC-producing *Enterobacteriaceae* on fresh vegetables in SA is limited. The aim of this exploratory study was to detect, identify, and characterize the AR of ESBL- and AmpC-producing *Enterobacteriaceae* isolates from frequently consumed fresh vegetables from selected retailing sites in Gauteng, the most densely populated province in SA.

Materials and Methods

Sample collection

A total number of 545 vegetable samples was collected from 10 formal retailers, 10 street trading greengrocers, 10 mobile trolley vendors, and 13 vendors at two farmers' markets in Gauteng, SA, from September 2017 to May 2018 (Supplementary Fig. S1). In the informal markets, street traders typically display fresh produce on a table, underneath a shade covering, at the roadside, or they use mobile trolleys. The vegetable samples included, depending on availability, spinach (bunches, baby leaves, or minimally processed ready-to-eat [RTE] pillow packs; *n*=200), tomatoes (*n*=200), cucumbers (*n*=45), lettuce (Iceberg lettuce heads or mixed salad leaf RTE pillow packs; *n*=50), and green beans (*n*=50 samples). All samples were transported in cooler boxes and stored at 4C until further processing within 24h.

Processing of fresh produce

At least three leaves from one spinach bunch and the inner leaves of three lettuce heads were used to prepare 50g composite samples of each of the leafy vegetable samples. Each spinach or lettuce sample was aseptically cut into a sterile polyethylene strainer stomacher bag containing 200mL buffered peptone water (BPW) (3M, Johannesburg, SA) in a 1:4 weight-to-volume ratio. A 150g sample of tomatoes and cucumbers (composite of at least three tomatoes or cucumbers) and a 150g sample of green beans were each placed into a sterile polyethylene stomacher bag containing 150mL BPW in a 1:1 weight-to-volume ratio (Xu *et al.*, 2015). Individual vegetable samples were blended for 5min at 230rpm in a Stomacher 400 circulator paddle blender (Seward Ltd., London, United Kingdom).

Isolation and identification of presumptive extended-spectrum and AmpC b-lactamase-producing Enterobacteriaceae

Each of the BPW–sample mixtures was incubated for 3–4 h at 37C after which 1 mL of each sample was added to 9 mL *Enterobacteriaceae* enrichment broth (Oxoid, Johannesburg, SA) according to ISO 21528-1:2004 and incubated overnight at 30C (Blaak *et al.*, 2014). ESBLproducing microorganisms were detected by streaking 10 lL of each of the enriched samples onto ChromID ESBL agar plates (bioMe´rieux, Midrand, SA) and incubated overnight at 30C (Blaak *et al.*, 2014). All presumptive positive ESBL/AmpC-producing *Enterobacteriaceae* colonies based on colony color, including weakly colored colonies, on the chromogenic media were isolated and purified.

Isolate identities were determined using matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF) (Bruker, Bremen, Germany) to species level as described by Standing *et al.* (2013). A single colony on nutrient agar was transferred to the MALDI-TOF polished steel target plate and further analyzed according to manufacturer's instructions (AOAC-OMA#2017.09), following calibration with the bacterial test standard. Non*Enterobacteriaceae* isolates were not included in further analysis.

Antimicrobial susceptibility testing

A selection of 77 presumptive ESBL-producing *Enterobacteriaceae* isolates, representing all unique species per product type from each supplier, were selected for further analysis. The Kirby-Bauer disk diffusion technique

was used to determine the resistance patterns of the isolates (Clinical Laboratory Standard Institute [CLSI], 2018). All isolates were screened for ESBL production by the double-disk synergy test (DDST) using cefotaxime 30lg, ceftazidime 30lg, and cefpodoxime 10lg, alone or in combination with clavulanic acid 10lg (Mast Diagnostics, Randburg, SA) (EUCAST, 2013). Zone diameters were compared with the CLSI and EUCAST criteria to determine if isolates were

resistant, intermediate, or susceptible. Isolates showing resistance to cefoxitin and cefotaxime or ceftazidime were regarded as a phenotypic indicator of AmpC production (EUCAST, 2013). Production of ESBLs was confirmed using the cefepime ESBL disc set (Cefepime 30lg, cefepimeclavulanic acid 30–10lg) and AmpC production using the AmpC detection set (Mast Diagnostics) (EUCAST, 2013; CLSI, 2018). Additional antimicrobials tested for resistance or susceptibility of isolates included ampicillin 10lg, amoxicillin-clavulanic acid 20/10lg, amoxicillin 10lg, trimethoprim-sulfamethoxazole 1.25/23.75 lg, imipenem 10lg, neomycin 10lg, tetracycline 30lg, gentamycin 10lg, chloramphenicol 10lg (Mast Diagnostics) (CLSI, 2018). Isolates resistant to three or more antimicrobial classes were regarded as MDR. *K. pneumoniae* ATCC 700603, *E. coli* NCTC 13315, *Enterobacter cloacae* NCTC 1406, and *E. coli* ATCC 25922 were included as positive and negative controls as described by the manufacturer (Mast Diagnostics).

Characterization of b-lactamase genes

The presence of ESBL determinants (bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{OXA}) and pAmpC resistance genes (bla_{ACC}, bla_{FOX}, bla_{MOX}, bla_{DHA}, bla_{CIT}, bla_{EBC}) in the selected isolates was analyzed with PCR and sequencing. Single colonies of each presumptive ESBL-producing *Enterobacteriaceae* isolate were cultured aerobically under shaking conditions at 200rpm in Tryptone soy broth (MERCK, Johannesburg, SA) for 24h at 30C. The cells were pelleted by centrifugation (12,500 *g* for 10min), DNA was extracted using the QuickgDNA Mini-Prep kit (Zymo Research, Irvine, CA), and the DNA concentration was determined using the Qubit dsDNA
Broad Range Assay and a Qubit 2.0 fluorometer (Life Technologies, Johannesburg, SA). PCR was performed using the DreamTaq Green PCR Master Mix (ThermoFisher Scientific, Johannesburg, SA), specific primers, and thermocycling conditions for each of the genes as described in Supplementary Table S1.

PCR products were sequenced using BigDye Terminator v3.1 cycle sequencing on an ABI 3500XL sequencer in forward and reverse directions (InquabaBiotec, Johannesburg, SA). The sequences were edited with Chromas 2.6 and BioEdit sequence alignment editor software, and consensus sequences were subjected to BLAST nucleotide search analysis to identify the AR genes.

Results

Identification of presumptive extended-spectrum and AmpC b-lactamase-producing Enterobacteriaceae isolates

Using MALDI-TOF analysis, 122 (28.2%) of the 432 presumptive extended-spectrum/AmpC b-lactamase-producing isolates obtained from the fresh vegetable samples were confirmed as *Enterobacteriaceae* belonging to 10 genera. The 310 non-*Enterobacteriaceae* isolates were predominantly identified as *Pseudomonas* spp. The *Enterobacteriaceae* isolates were identified as *Enterobacter* spp. (28.7%), including *E. cloacae*, *Enterobacter asburiae*, *Enterobacter cowanii*, and *Enterobacter ludwigii*; *Serratia* (18.9%), including predominantly *Serratia fonticola*; *E. coli* (18%); *Klebsiella* spp. (14.8%), including *K. pneumoniae* and *Klebsiella oxytoca*; *Rahnella aquatilis* (9%); *Proteus* spp. (4.9%), including *Proteus penneri* and *Proteus mirabilis*; *Citrobacter* spp. (2.5%), including *Citrobacter farmeri* and *C. freundii*; *Kluyvera ascorbata* (1.64%); *Achromobacter xylosixidans* (1.6%); and *Raoultella ornithinolytica* (0.8%). Presumptive ESBL/AmpC-producing *Enterobacteriaceae* were isolated from the vegetable types tested.

Phenotypic AR profiling

All the 77 selected presumptive ESBL-producing *Enterobacteriaceae* showed resistance to more than one antimicrobial agent, with 96.1% being MDR (resistant to ‡3 antimicrobial classes) (Fig. 1). Resistance to the aminoglycosideandchloramphenicolclasseswasdominant,observedin
94.8%and85.7%oftheisolates,respectively.Allisolateswith cephalosporin resistance (CTX30C, CAZ30C, CPD10C, or CPM30C) were further screened using DDST, after which 61/ 77 (79.2%) were tested positive for ESBL production (Fig. 1). All isolates that showed cefoxitin resistance (*n*=46) were
additionallyscreenedwiththeAmpCdetectionset.Fromthese 46 isolates, 32/77 (41.6%) were tested positive for AmpC production. This included 27 isolates showing resistance to cefoxitin, ceftazidime, and/or cefotaxime and additionally five isolates that showed cefoxitin resistance, but ceftazidime and/or cefotaxime susceptibility. All isolates displaying ESBL or AmpC phenotypes were further characterized for the identification of ESBL and/or AmpC resistance genes.

Genotypic AR profiling

Genes encoding b-lactamases were detected in 58/77 (75.3%) isolates obtained from all vegetable types, mainly in *E. coli* (*n*=20), *Enterobacter* spp. (*n*=12), and *Serratia* spp. (*n*=11) isolates. This included 37 (48%) broad-spectrum, 39 (51%) ESBL, and 20 (25.9%) AmpC genetic determinants (Fig. 1). The most frequently detected b-lactamase genes were bla_{CTX-M} (*n*=28), followed by bla_{SHV} (*n*=22), bla_{TEM} (*n*=21), and bla_{OXA} (*n*=5). ESBLs encoded by bla_{CTX-M} included CTX-M-14 (*n*=15), CTX-M-15 (*n*=6), CTX-M-27 (*n*=4), and CTX-M-55 (*n*=3); bla_{TEM} genes encoded TEM3 (*n*=3), while bla_{SHV} genes encoded SHV-18

(*n*=6), SHV28 (*n*=1), and SHV-154 (*n*=1). All the *bla*$_{OXA}$, 85.7% (*n*=18) of the *bla*$_{TEM}$, and 63.6% (*n*=14) of the *bla*$_{SHV}$

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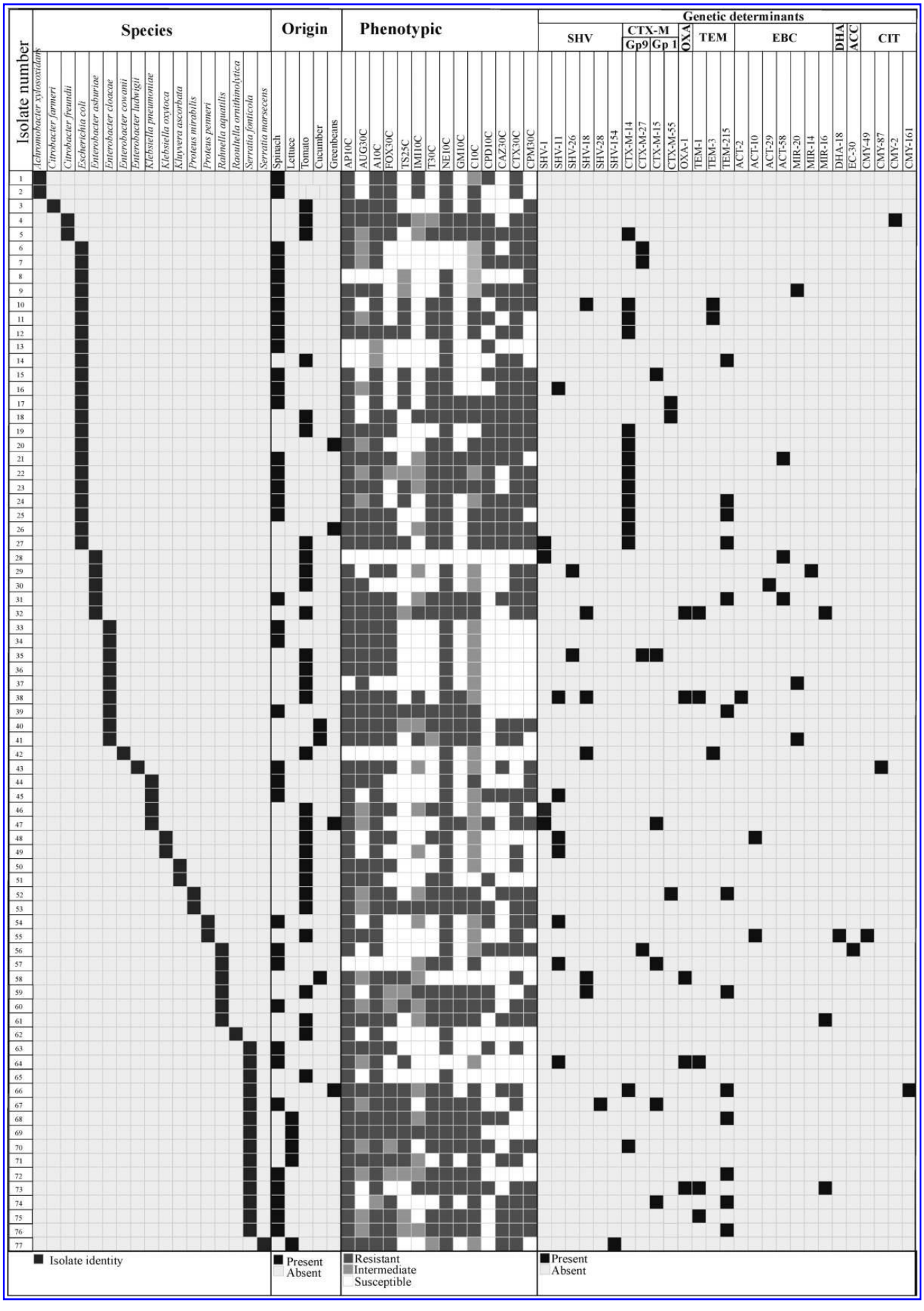
FIG. 1. Summary of the species isolated from different fresh vegetables, indicating the phenotypic resistance profiles and the extended-spectrum b-lactamase/AmpC genetic determinants detected. The color code is given in the lower left corner of each section in grayscale: species identification (dark grey); isolate origin (black); phenotypic antimicrobial resistance—resistant (grey), intermediate resistant (light grey), or susceptible (white); genotypic determinants (black). AP10C, ampicillin; AUG30C, amoxicillin-clavulanic acid; A10C, amoxicillin; FOX30C, cefoxitin; CPM30C, cefepime; CPD10C, cefpodoxime; CPD10C/ CLAV1C, cefpodoxime-clavulanic acid; CAZ30C, ceftazidime; CAZ/CLAV10C, ceftazidime-clavulanic acid; CTX30C, cefotaxime; CTX/CLAV10C, cefotaxime-clavulanic acid; TS25C, trimethoprim-sulfamethoxazole; IMI10C, imipenem; T30C, tetracycline; NE10C, neomycin; C10C, chloramphenicol.


Isolate number
Species
Origin
Phenotypic
Genetic determinants
SHV
CTX-M
Gp9
Gp 1
OXA
TEM
EBC
DHA
ACC
CIT
Achromobacter xylosoxidans
Citrobacter farmeri
Citrobacter freundii
Escherichia coli
Enterobacter asburiae
Enterobacter cloacae
Enterobacter cowanii
Enterobacter ludwigii
Klebsiella pneumoniae
Klebsiella oxytoca
Kluyvera ascorbata
Proteus mirabilis
Proteus penneri
Rahnella aquatilis
Raoultella ornithinolytica
Serratia fonticola
Serratia marcescens
Spinach
Lettuce
Tomato
Cucumber
Greenbeans
AP10C
AUG30C
A10C
FOX30C
TS25C
IMI10C
T30C
NE10C
GM10C
C10C
CPD10C
CAZ30C
CTX30C
CPM30C
SHV-1
SHV-11
SHV-26
SHV-18
SHV-28
SHV-154
CTX-M-14
CTX-M-27
CTX-M-15
CTX-M-55
OXA-1
TEM-1
TEM-3
TEM-215
ACT-2
ACT-10
ACT-29
ACT-58
MIR-20
MIR-14
MIR-16
DHA-18
EC-30
CMY-49
CMY-87
CMY-2
CMY-161
Isolate identity
Present
Absent
Resistant
Intermediate
Susceptible
Present
Absent

sequences encoded broad-spectrum b-lactamases OXA-1, TEM-1, TEM-215, SHV-1, SHV-11, or SHV-26, respectively. Three isolates harbored more than one ESBL; one

E. coli isolate carried the $bla_{\text{TEM-3}}$, $bla_{\text{SHV-18}}$, and $bla_{\text{CTX-M14}}$ genes, and two isolates (*E. coli* and *E. cowanii*) carried the $bla_{\text{TEM-3}}$ gene in association with $bla_{\text{CTX-M-14}}$ and $bla_{\text{SHV-18}}$ genes, respectively. In 12 isolates (*E. coli* [*n*=3]; *Enterobacter* spp. [*n*=3]; *Serratia* spp. [*n*=3]; *R. aquatilis* [*n*=2]; and *P. mirabilis* [*n*=1]), ESBL genes in association with broad-spectrum b-lactamases were detected (Fig. 1).

AmpC resistance genes were detected in 18/58 (31%) isolates harboring b-lactamase genetic determinants (Fig. 1). In 17 isolates, only one pAmpC genetic determinant was detected; $bla_{\text{MIR-20}}$ (*n*=4), $bla_{\text{MIR-16}}$ (*n*=3), $bla_{\text{ACT-58}}$ (*n*=2), and one isolate each carried $bla_{\text{CMY-2}}$, $bla_{\text{MIR-14}}$, $bla_{\text{ACT-29}}$, $bla_{\text{ACT-10}}$, $bla_{\text{ACT-2}}$, bla_{EC}, $bla_{\text{CMY-161}}$, or $bla_{\text{CMY-87}}$ respectively. Among these 17 isolates, five isolates (*Enterobacter* spp. [*n*=2], *E. coli* [*n*=1], *R. aquatilis* [*n*=1], and *S. fonticola* [*n*=1]) also harbored ESBL genetic determinants. One *P. penneri* isolate carried three AmpC genes

(bla_{ACT10}, $bla_{\text{DHA-18}}$, and $bla_{\text{CMY-49}}$). The EBC family of the AmpC genetic determinants was the most dominant type.

Discussion

MDR ESBL/AmpC-producing *Enterobacteriaceae* were detected for the first time in raw vegetables retailed at selected sites in Gauteng Province, SA. Antibiotic-resistant opportunistic pathogens on fresh produce are a serious health concern that contributes toward the burden of AR in different environments, leading to increased risk of infection if colonization in humans occurs (Al-Kharousi *et al.*, 2016). *Enterobacteriaceae* regarded as emerging bacterial threats include *E. coli*, *K. pneumoniae*, and *Enterobacter* spp. showing resistance to blactams and aminoglycosides (Fair and Tor, 2014).

Presumptive ESBL producers, predominantly *E. coli*, *K. pneumoniae*, *E. cloacae, and E. asburiae*, were detected in 17.4% of our vegetable samples analyzed. This is lower than the 25.4% reported by Zurfluh *et al.* (2015) for imported vegetables into Switzerland from the Dominican Republic, India, Thailand, and Vietnam, but higher than the 6% reported by Reuland *et al.* (2014) on retail vegetables in the Netherlands. Similar to Blaak *et al.* (2014), environmental ESBL-producing *Enterobacteriaceae* isolated from vegetables included *S. fonticola* and *R. aquatilis*.

Phenotypic confirmation of ESBL/AmpC production showedthat61(79.9%)ofthe77analyzed*Enterobacteriaceae* isolates displayed an ESBL-producing phenotype and 41.6% an AmpC-producing phenotype, which is higher than results reported by van Hoek *et al.* (2015). Combined ESBL- and AmpC-producingphenotypeswerealsoobservedin35%ofthe isolates. MDR phenotypes (resistance to ‡3 antimicrobial classes) were observed in 96.1% of our analyzed isolates. The most prevalent non-b-lactam resistance profiles showed resistance against aminoglycoside (94.8%), chloramphenicol (85.7%), and tetracycline (53.2%). This is higher than reports fromsimilarstudiesthatshowedresistancetoaminoglycosides (46.7–66.7%), chloramphenicol (33.3%) (Zurfluh *et al.*, 2015; Ben Said *et al.*, 2016), and tetracycline (46.7%) (Ben Said *et al.*, 2016) in ESBL-producing *Enterobacteriaceae*.

Genes expressing broad-spectrum b-lactamases, ESBLs, and/or AmpC b-lactamases were detected in 69.9% of our MDR isolates. Co-expression of ESBL and AmpC genes in environmental (van Hoek *et al.*, 2015; Ye *et al.*, 2017) and clinical (Tau *et al.*, 2012; Kharat *et al.*, 2017) *Enterobacteriaceae* isolates has also been reported. Globally the $bla_{\text{CTX-M-type}}$ ESBL genes are predominant in *Enterobacteriaceae,* which was similar in our study, the majority being detected in *E. coli* isolates. $bla_{\text{CTX-M-14}}$ was the main genetic determinant detected from mostly *E. coli* and *C. freundii* isolates, which corresponds to results obtained from vegetable samples in Tunisia (Ben Said *et al.*, 2016). Isolates harboring $bla_{\text{CTX-M-15}}$ included *E. coli*, *E. cloacae*, *K. pneumoniae*, *R. aqualtilis*, and *S. fonticola* and were second most prevalent in our study. $bla_{\text{CTX-M-15}}$ was the most prevalent gene detected in *E. coli* and *K. pneumoniae* isolates from fresh vegetables imported into Switzerland from India and the Dominican Republic (Zurfluh *et al.*, 2015). This is in agreement with reports that $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ are predominant and have been associated with clinically relevant *Enterobacteriaceae* infections (Ehlers *et al.*, 2009; Zurfluh *et al.*, 2015).

In contrast to Njage and Buys (2014), who predominantly detected $bla_{\text{CTX-M Group 8/25}}$-positive *E. coli* isolates from lettuce in the North West Province (SA), no $bla_{\text{CTX-M Group 8/ 25}}$ genes were detected in any of our *E. coli* isolates from the vegetable samples analyzed. The $bla_{\text{CTX-M-15}}$ (CTX-M group 1) and $bla_{\text{CTX-M-14}}$ (CTX-M group 9) genes detected in our environmental isolates, reported to be closely related to chromosomally encoded bla_{FONA} and bla_{RAHN} genes of *S. fonticola* and *R. aquatilis*, had no significant similarity in the GenBank database using NCBI BLAST based on total BLAST alignment scores. This contrasts results reported by Raphael *et al.* (2011) where sequences similar to $bla_{\text{RAHN-2}}$ and $bla_{\text{FONA-5}}$ were detected using $bla_{\text{CTX-M}}$ primers.

In our study, five isolates, including *E. coli*, *Enterobacter* spp., *R. aquatilis*, *S. fonticola*, simultaneously harbored ESBL and AmpC genes. Environmental isolates are known to carry chromosomally encoded AmpC b-lactamases. However, *Enterobacteriaceae* harboring both chromosomal and pAmpC b-lactamases are increasingly reported to hydrolyze broad-spectrum cephalosporins more efficiently, resulting in adverse treatment options in clinical settings (Jacoby, 2009; Reuland *et al.*, 2014).

The 18 isolates in which pAmpC resistance genes were detected predominantly included the EBC-type pAmpC blactamases (identified as bla_{ACT}/bla_{MIR}). This contrasts with two previous studies where bla_{CIT}, bla_{DHA}, or bla_{ACC} pAmpC b-lactamases were mostly detected in *Enterobacteriaceae* isolated from fresh produce and water samples (Njage and Buys, 2014; Ye *et al.*, 2017). $bla_{ACT/MIR}$ genes have been reported tobe thedominantAmpCgenetic determinantsin*Enterobacter* spp., causing intra-abdominal infections (Khari *et al.*, 2016), and were detected in seven of the *Enterobacter* spp. isolates in our study. The fact that fresh produce can serve as a reservoir of MDR ESBL/AmpC-producing *Enterobacteriaceae*, including their genetic determinants, constitutes a potential health risk to the consumer as resistance to antimicrobials frequently used to treat human infections was shown.

Conclusion

The results obtained from screening at these selected sites indicate that further investigation of different fresh produce types in Gauteng and other provinces in SA is necessary. Future studies should focus on the surveillance of production systems from farm to retail to identify potential sources of contamination that contribute to the presence and dissemination of antimicrobial-resistant microorganisms and their genetic determinants. Since AR is a worldwide problem, a global solution is required that integrates the contributions from government departments as well as from the scientific community.

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Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Figure S1
Supplementary Table S1

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